

Evidence of a biochemical
interaction between insect and specific
foodplant in the system
Parnassius apollo-*Sedum album*

by

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With 5 figures

ABSTRACT

An interaction involving a host-plant produced insect-hostile factor, operating in the system *Parnassius apollo* — *Sedum album*, is described. Insect-hostility of natural foodplant material collected periodically in the low-altitude Jura region is at maximum in midwinter. First-generation larvae derived from the alpine *Apollo* race are more sensitive to the inadequacies of winter-*Sedum* foodplant material than are larvae derived from the Jura *Apollo* race. It is not ascertained yet whether short-day photoperiod alone or combined with cold, or reduced total daily photosynthesis is responsible for the increased insect-hostility of the *Sedum* foodplant in midwinter.

1. INTRODUCTION

One of the most fascinating tasks of modern ecology is the search for biochemical interactions between herbivorous insects and their foodplants (HARBORNE *et al.* 1977; DALY *et al.* 1978; WALLACE *et al.* 1975). The identification of such interactions and the combined study of their dynamics, by interdisciplinary approaches, in natural and in artificial physical environments, primarily yield fundamental insight into ecosystems-dynamic and -evolution; but they also inspire new approaches and concepts in a variety of technological tasks, such as for example pest control or nature conservation.

The objective of this preliminary communication is to report on an (possibly allelo-chemic) interaction which has been observed by us in the system *Parnassius apollo*-*Sedum album* and which involves a hostplant produced insect-hostile factor.

While attempting all-year-round breeding of *Parnassius* butterflies in the laboratory with a food supply of *Sedum album* collected periodically in the field it was observed that the mortality rate of larvae and pupae increased drastically in December, reaching frequently 100% or nearly 100% for insects raised in January and February. In March, the mortality decreased again to the level characteristic for the autumn months. Based on this interesting observation a series of experiments were carried out in order to determine whether the growing condition of the natural foodplant or the state of activated egg larvae is responsible for the increased insect mortality observed in midwinter.

2. DESCRIPTION OF RELEVANT ECOSYSTEMS COMPONENTS

Parnassius apollo is a univoltine (1 generation per year) specialist phytophagous insect with palaearctic distribution in mountainous areas of Europe and Western Asia. (BRYK 1935). In its natural biotopes — which are sun exposed warm stony slopes or rock faces normally at altitudes ranging from 500 to 1 600 meters above sea level in central Europe — this butterfly breeds during the summer months (June to September). Embryonic development starts immediately after fertilization of the egg and is completed within a week or more, leaving the vast majority of fully developed L1 enclosed in the egg shell. These L1 then enter a diapause normally lasting from 4 to 6 months and requiring cold for its successful termination in midwinter. The majority of eggs hatch in the spring when the snow melts and the temperature rises above 0° C, that is in March or April, depending upon the climatic conditions prevailing in the particular biotope. Exceptional L1 hatches have been observed in early or late autumn, and, at lower altitudes in snow-free places, are likely to occur also on warm winter days (see figure 5). The postembryonic development of this butterfly consists of 5 larval instars.

Sedum album, the white stonecrop (Hess *et al.* 1978), is the specific foodplant of most of the central and south European geographic races of *Parnassius apollo*. *Sedum album* is an evergreen perennial plant with palaearctic distribution. In its natural environment it flowers during the summer months from June to September, depending on altitude and local climatic conditions. Though this plant's photoperiodic response characteristics are not known yet, *Sedum album* is most likely a long-day plant species requiring cold for flower induction. The North European, Carpathian, North Russian and Siberian geographic races of *Parnassius apollo* are adapted to *Sedum telephium* which is a long-day plant species not requiring cold for flower induction (VINCE-PRUE 1975). Recently our own experiments have shown that *Parnassius apollo* larvae of alpine origin (Lötschental, Wallis) grow equally well on *Sedum telephium* as on their natural foodplant, *Sedum album*.

3. MATERIALS AND METHODS

3.1 Insect

The insect material was derived from two different regions and altitudes, namely

- a) biotopes situated in the Swiss Alps at altitudes between 1 500 to 1 600 m above sea level (Lötschental, Binntal): Geographic races *Paranassius apollo geminus* and *valesiacus*;

- b) biotopes situated in the Swiss Jura at an altitude of 650 m above sea level (Vallon de St. Imier): Geographic race *Parnassius apollo nivatus*.

During the peak summer months females were caught and brought to oviposition separately in airy gauze cages in the laboratory at a temperature of 25 to 27° C and under a light intensity of a few thousand Lux. Daily feeding with 10% American-mountain-flower-honey water yielded, over a period of 2-3 weeks, from 100 to 250 eggs per female. The harvested eggs were subsequently stored in linnen covered transparent plastic vessels over a water base, first for 2-3 weeks at 25° C, then for 1-2 weeks at 15° C and subsequently at 4° C either under light with a photoperiod of 19 hours or in the dark, until used for the tests. By this method it was possible to obtain samples of viable first-generation L1 larvae for testing at any time between mid December and mid April, by activation of eggs at elevated temperatures (15° C or 25° C) with an obligatory daily light phase of 16-19 hours. Test material for the few autumn experiments was secured in part by the exceptional early spontaneous egg hatches, or by surviving *Parnassius apollo valesiacus* eggs stored at an average temperature of -2.5° C in the dark for 15 months¹.

Except for preliminary or special tests, indoor-breeding of activated L1 larvae was performed in single cultures in transparent plastic vessels and with a daily supply of cuts from fresh foodplants or from stored foodplant material. The cultures were kept in climatized systems (green house, climatic chamber) with a constant air temperature ranging from 22 to 27° C, a relative humidity in the circulating air of 60% to 80%, and under mercury vapour lamps producing constant light intensities ranging from 3 000 to 9 000 Lux on the culture surface. A specified daily light cycle ranging from 16 to 24 hours was maintained. A detailed description of our methods of controlled breeding of European *Parnassius* butterflies together with exact estimations of developmental parameters will be presented later.

3.2 Food plant

The *Sedum album* was derived almost exclusively from the Swiss Jura (Basel — Porrentruy region) and from altitudes of between 300 m and 600 m above sea level. *Sedum telephium* was derived from seeds obtained by the Botanical Institute. For a number of *Apollo* tests *Sedum album* was grown indoors under long-day conditions (photoperiod 16-19 hours = LD) or under short-day conditions (photoperiod 8-10 hours = SD) either in hydroculture with Hoagland's nutrient solution or in pots. During the light cycles, maintained with light intensities ranging from several thousand to 35 000 Lux, the air temperature was set at from 22 to 27° C. *Sedum telephium*, used in only one of the *Apollo* tests, was grown in greenhouse pot cultures under long-day conditions. Indoorcultivation of the *Sedum plants* under long-day conditions and their subsequent use as nutrient source in control experiments was often interfered with by extensive aphid infestations. Since it was found that such infestations apparently convert optimal foodplant into an ineffective and detrimental nutrient source for *Apollo* larvae, heavily infested *Sedum cultures* were excluded as test material. Natural *Sedum album* foodplant batches collected outdoors at various times of the year were used either immediately or following storage in the dark, at 4° C for 1 to 2 weeks or at -5° C for longer periods.

¹ Jura-*Apollo* derived eggs stored under the same conditions survive only for 10 months.

3.3 Evaluation of data

For the biometric evaluation of data the following definitions were made:

- N_0 = Initial test-sample size; the initial number of L1 larvae used in a particular experiment.
- $E(K)$ = Total number of emergences with respect to a specific instar IK (= L2, L3, L4, L5, I6 (PUPA), I7 (IMAGO)) i.e. total number of instars IK produced by moults $M(K-1)$. For instar IK = I1 (= L1), $E(1) = N_0$.
- $E(K)\%$ = $100 \cdot E(K)/N_0$.
- $D(K)$ = Total number of deaths which have occurred in instar IK: Cumulative mortality of instar IK ($K = 1, 2, \dots, 7$).
- $D(K)\%$ = $100 \cdot D(K)/N_0$.
- $C_M(K)$ = Specific mortality coefficient of instar IK ($K = 1, 2, \dots, 7$), $C_M(K) = D(K)/E(K) \equiv D(K)\%/E(K)\%$;
 $C_M(K)$ defines the fraction, of the number $E(K)$ of individuals entering instar IK (by moult $M(K-1)$ or otherwise), which subsequently die during this and only this particular instar IK.
- 'NOR' = An index label referring to normal (perfect) moults i.e. entries into a given instar.
- 'DEF' = An index label referring to defective (imperfect) moults i.e. entries into instars. (With respect to imaginal moults it also refers to total or partial incapacity of unfolding wings following emergence from the pupal exuvia).
- 'TOT' = An index label referring to the total 'NOR + DEF'.
- 'M' = An index label referring to male insects.
- 'F' = An index label referring to female insects.
- $N_{0(TOT)}$ = Total number of L1 larvae tested in a set of (pooled) experiments.
- MIN. = Observed minimum value in N_{EXP} (pooled) experiments.
- MAX. = Observed maximum value in N_{EXP} (pooled) experiments.
- STDM. = Standard deviation of mean value of N_{EXP} (pooled) experiments.

4. RESULTS

4.1 Response of Apollo larvae to indoor-grown long-day foodplant

LD laboratory cultures of *Sedum* plants served as suitable reference foodplant-materials for our control experiments with *Apollo* larvae. Up to now we have carried out eight *Apollo* tests with *Sedum* plants which were cultured indoors under controlled LD conditions, seven of them with *Sedum album* and one with flowering *Sedum telephium*. The results are listed in table I and illustrated in figure 1. In all these control experiments the foodplant was grown under LD for at least 2 months prior to the insect tests; and the newly hatched L1 used for the tests were derived exclusively from F_1 egg populations with hatchabilities of not less than 70%. The tests covered the period from mid December to end of May.

Growth of L1 on indoor-cultured LD *Sedum album* foodplant yielded in the average 82% normally developed imagoes. Our data do not indicate a differential response of the Jura and the alpine race of *Parnassius apollo* to the cultured LD plant. The mortality profiles of controls, displayed in figures 3 and 4 show that the instar-specific insect mortalities on indoor-grown LD *Sedum album* remain small during all five larval instars and during the pupal stage, not exceeding 10%. A comparable response characteristic

TABLE I. — Response of *Parnassius apollo* larvae to *Sedum album* foodplant material derived from long-day indoor cultures : Developmental score of larvae derived from the Jura- and alpine race (controls). Updated 22.9.79.

Test-insect	Number of experiments N _{EXP}	Total number of tested larvae N _{0(TOT)}	Foodplant	Response of test insect: Moults i.e. emergencies (% of N ₀)							
				Moult 1 (L1 → L2)	Moult 2 (L2 → L3)	Moult 3 (L3 → L4)	Moult 4 (L4 → L5)	Moult 5 (L5 → PUPA)	Moult 6 (PUPA → IMAGO)	Moult 6M (PUPA → MALE IMAGO)	Moult 6F (PUPA → FEMALE IMAGO)
<i>Parnassius apollo</i> : Alpine race	2	7	<i>Sedum album</i> long-day laboratory culture	TOT	MEAN = MIN. = MAX. = STDM. =	90.00 80.00 100.00 10.00	90.00 80.00 100.00 10.00	90.00 80.00 100.00 10.00	90.00 80.00 100.00 10.00	90.00 80.00 100.00 10.00	0.00 0.00 0.00 0.00
				NOR	MEAN = MIN. = MAX. = STDM. =	90.00 80.00 100.00 10.00	90.00 80.00 100.00 10.00	90.00 80.00 100.00 10.00	90.00 80.00 100.00 10.00	90.00 80.00 100.00 10.00	0.00 0.00 0.00 0.00
				DEF	MEAN = MIN. = MAX. = STDM. =	0.0 0.0 0.0 0.0	0.0 0.0 0.0 0.0	0.0 0.0 0.0 0.0	0.0 0.0 0.0 0.0	0.0 0.0 0.0 0.0	0.0 0.0 0.0 0.0
				TOT	MEAN = MIN. = MAX. = STDM. =	97.50 87.50 100.00 2.50	95.00 87.50 100.00 3.06	90.00 62.50 100.00 7.29	86.07 62.50 100.00 7.46	48.48 35.71 66.67 6.64	30.81 0.0 60.00 9.66
<i>Parnassius apollo</i> : Jura race	5	38	<i>Sedum album</i> long-day laboratory culture	NOR	MEAN = MIN. = MAX. = STDM. =	97.50 87.50 100.00 2.50	95.00 87.50 100.00 3.06	90.00 62.50 100.00 7.29	83.21 62.50 100.00 7.35	48.48 35.71 66.67 6.64	30.81 0.0 60.00 9.66
				DEF	MEAN = MIN. = MAX. = STDM. =	0.0 0.0 0.0 0.0	0.0 0.0 0.0 0.0	0.0 0.0 0.0 0.0	0.0 0.0 14.29 2.86	0.0 0.0 0.0 0.0	0.0 0.0 0.0 0.0
				TOT	MEAN = MIN. = MAX. = STDM. =	100.00 100.00 100.00 0.0	100.00 100.00 100.00 0.0	100.00 100.00 100.00 0.0	100.00 75.00 100.00 2.86	75.00 50.00 100.00 0.0	25.00 25.00 100.00 0.0
				NOR	MEAN = MIN. = MAX. = STDM. =	100.00 100.00 100.00 0.0	100.00 100.00 100.00 0.0	100.00 100.00 100.00 0.0	100.00 75.00 100.00 2.86	75.00 50.00 100.00 0.0	25.00 25.00 100.00 0.0

TABLE II
*Response of Parnassius apollo larvae to (natural) Sedum album foodplant material collected periodically in the field ;
 Developmental score of larvae derived from the alpine race.*

Test-insect	Number of experiments N _{EXP}	Total number of tested larvae N _{OTOT}	Sedum album-foodplant field-collection period		Response of test insect: Moults i.e. emergencies (% of N ₀)							
					Moult 1 (L1 → L2)	Moult 2 (L2 → L3)	Moult 3 (L3 → L4)	Moult 4 (L4 → L5)	Moult 5 (L5 → PUPA)	Moult 6 (PUPA → IMAGO)	Moult 6M (PUPA → MALE IMAGO)	Moult 6F (PUPA → FEMALE IMAGO)
<i>Parnassius apollo</i> : Alpine race	2	15	Autumn : September, 18 to November, 7	TOT	MEAN = MIN. = MAX. = STDM. =	100.00 100.00 100.00 0.0	100.00 100.00 100.00 0.0	100.00 100.00 100.00 0.0	92.86 85.71 100.00 7.14	86.61 85.72 87.50 0.89	45.54 28.57 62.50 16.96	41.07 25.00 57.15 16.07
				NOR	MEAN = MIN. = MAX. = STDM. =	100.00 100.00 100.00 0.0	100.00 100.00 100.00 0.0	100.00 100.00 100.00 0.0	92.86 85.71 100.00 7.14	73.21 71.43 75.00 1.78	39.29 28.57 50.00 10.71	33.93 25.00 42.86 8.93
				DEF	MEAN = MIN. = MAX. = STDM. =	0.0 0.0 0.0 0.0	0.0 0.0 0.0 0.0	0.0 0.0 0.0 0.0	0.0 0.0 0.0 0.0	13.39 12.50 14.29 0.89	6.25 0.0 12.50 6.25	7.15 0.0 14.29 7.14

3	30	Winter: December, 31 to February, 26	TOT	MEAN =	100.00	100.00	100.00	100.00	86.67	63.33	13.33	13.33	0.0
				MIN. =	100.00	100.00	100.00	100.00	60.00	20.00	0.0	0.0	0.0
				MAX. =	100.00	100.00	100.00	100.00	100.00	100.00	30.00	30.00	0.0
				STD.M. =	0.0	0.0	0.0	0.0	13.33	23.33	8.82	8.82	0.0
			NOR	MEAN =	100.00	100.00	100.00	100.00	80.00	63.33	10.00	10.00	0.0
				MIN. =	100.00	100.00	100.00	100.00	50.00	20.00	0.0	0.0	0.0
				MAX. =	100.00	100.00	100.00	100.00	100.00	100.00	30.00	30.00	0.0
				STD.M. =	0.0	0.0	0.0	0.0	15.28	23.33	10.00	10.00	0.0
			DEF	MEAN =	0.0	0.0	0.0	0.0	6.67	0.0	3.33	3.33	0.0
				MIN. =	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
				MAX. =	0.0	0.0	0.0	0.0	10.00	0.0	10.00	10.00	0.0
				STD.M. =	0.0	0.0	0.0	0.0	3.33	0.0	3.33	3.33	0.0
4	44	Spring: April, 4 to May, 12	TOT	MEAN =	98.44	98.44	98.44	98.44	98.44	92.19	89.06	35.46	53.59
				MIN. =	93.75	93.75	93.75	93.75	93.75	68.75	56.25	14.29	37.50
				MAX. =	100.00	100.00	100.00	100.00	100.00	100.00	100.00	58.82	85.71
				STD.M. =	1.56	1.56	1.56	1.56	1.56	7.81	10.94	11.12	11.02
			NOR	MEAN =	98.44	98.44	98.44	98.44	98.44	90.63	86.12	35.46	50.65
				MIN. =	93.75	93.75	93.75	93.75	93.75	62.50	56.25	14.29	29.41
				MAX. =	100.00	100.00	100.00	100.00	100.00	100.00	100.00	58.82	85.71
				STD.M. =	1.56	1.56	1.56	1.56	1.56	9.38	10.34	11.12	12.43
			DEF	MEAN =	0.0	0.0	0.0	0.0	0.0	1.56	2.94	0.0	2.94
				MIN. =	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
				MAX. =	0.0	0.0	0.0	0.0	0.0	6.25	11.76	0.0	11.76
				STD.M. =	0.0	0.0	0.0	0.0	0.0	1.56	2.94	0.0	2.94

TABLE III. — *Response of Parnassius apollo larvae to (natural) Sedum album foodplant material collected periodically in the field : Developmental score of larvae derived from the Jura race.*

Test-insect	Number of experiments N _{EXP}	Total number of tested larvae N ₀ (TOT)	Sedum album-foodplant field-collection period	Response of test insect: Moults i.e. emergencies (% of N ₀)								
				Moult 1 (L1 → L2)	Moult 2 (L2 → L3)	Moult 3 (L3 → L4)	Moult 4 (L4 → L5)	Moult 5 (L5 → PUPA)	Moult 6 (PUPA → IMAGO)	Moult 6M (PUPA → MALE IMAGO)	Moult 6F (PUPA → FEMALE IMAGO)	
<i>Parnassius apollo</i> : Jura race	4	46	Winter: December, 31 to February, 15	TOT	MEAN = MIN. = MAX. = STDM. =	100.00 100.00 100.00 0.0	94.05 76.19 100.00 5.95	89.28 57.14 100.00 10.71	86.90 47.62 100.00 13.09	64.73 0.0 100.00 22.36	50.54 0.0 75.00 17.26	25.09 0.0 42.86 9.68
				NOR	MEAN = MIN. = MAX. = STDM. =	100.00 100.00 100.00 0.0	94.05 76.19 100.00 5.95	89.28 57.14 100.00 10.71	86.90 47.62 100.00 13.09	58.04 0.0 100.00 21.25	50.54 0.0 75.00 17.26	25.09 0.0 42.86 9.68
				DEF	MEAN = MIN. = MAX. = STDM. =	0.0 0.0 0.0 0.0	0.0 0.0 0.0 0.0	0.0 0.0 0.0 0.0	0.0 0.0 0.0 0.0	6.70 0.0 14.29 3.88	0.0 0.0 0.0 0.0	0.0 0.0 0.0 0.0
				TOT	MEAN = MIN. = MAX. = STDM. =	100.00 100.00 100.00 0.0	100.00 100.00 100.00 0.0	100.00 100.00 100.00 0.0	100.00 100.00 100.00 0.0	85.00 70.00 100.00 15.00	70.00 60.00 80.00 10.00	25.00 20.00 30.00 5.00
				NOR	MEAN = MIN. = MAX. = STDM. =	100.00 100.00 100.00 0.0	100.00 100.00 100.00 0.0	100.00 100.00 100.00 0.0	100.00 100.00 100.00 0.0	85.00 70.00 100.00 15.00	45.00 40.00 50.00 5.00	10.00 0.0 20.00 10.00
				DEF	MEAN = MIN. = MAX. = STDM. =	0.0 0.0 0.0 0.0	0.0 0.0 0.0 0.0	0.0 0.0 0.0 0.0	0.0 0.0 0.0 0.0	0.0 0.0 0.0 0.0	25.00 10.00 20.00 10.00	15.00 0.0 0.0 5.00
	2	15	Spring: April, 15 to April, 23	TOT	MEAN = MIN. = MAX. = STDM. =	100.00 100.00 100.00 0.0	100.00 100.00 100.00 0.0	100.00 100.00 100.00 0.0	100.00 100.00 100.00 0.0	85.00 70.00 100.00 15.00	70.00 60.00 80.00 10.00	25.00 30.00 30.00 5.00
				NOR	MEAN = MIN. = MAX. = STDM. =	100.00 100.00 100.00 0.0	100.00 100.00 100.00 0.0	100.00 100.00 100.00 0.0	100.00 100.00 100.00 0.0	85.00 70.00 100.00 15.00	45.00 40.00 50.00 5.00	10.00 0.0 20.00 10.00
				DEF	MEAN = MIN. = MAX. = STDM. =	0.0 0.0 0.0 0.0	0.0 0.0 0.0 0.0	0.0 0.0 0.0 0.0	0.0 0.0 0.0 0.0	0.0 0.0 0.0 0.0	25.00 10.00 20.00 10.00	15.00 0.0 0.0 5.00
				TOT	MEAN = MIN. = MAX. = STDM. =	100.00 100.00 100.00 0.0	100.00 100.00 100.00 0.0	100.00 100.00 100.00 0.0	100.00 100.00 100.00 0.0	85.00 70.00 100.00 15.00	70.00 60.00 80.00 10.00	25.00 30.00 30.00 5.00
				NOR	MEAN = MIN. = MAX. = STDM. =	100.00 100.00 100.00 0.0	100.00 100.00 100.00 0.0	100.00 100.00 100.00 0.0	100.00 100.00 100.00 0.0	85.00 70.00 100.00 15.00	45.00 40.00 50.00 5.00	10.00 0.0 20.00 10.00
				DEF	MEAN = MIN. = MAX. = STDM. =	0.0 0.0 0.0 0.0	0.0 0.0 0.0 0.0	0.0 0.0 0.0 0.0	0.0 0.0 0.0 0.0	0.0 0.0 0.0 0.0	25.00 10.00 20.00 10.00	15.00 0.0 0.0 5.00

is obtained with the substitute foodplant, *Sedum telephium*, grown indoors under LD conditions (see Table I). In the majority of these control experiments the size of the emerging male and female adults was comparable to that which is characteristic of imagoes from the natural populations of the Jura- and alpine biotopes.

Our control experiments with LD laboratory cultures of *Sedum* permitted assessment of possible effects of the age (i.e. state), of activated egg larvae, on their characteristic

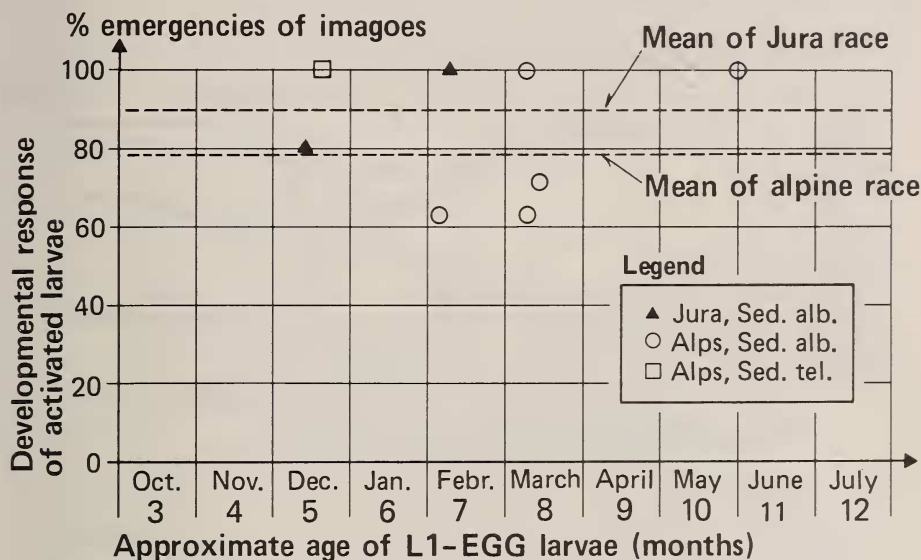


FIG. 1.

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Developmental response of *Parnassius apollo* post-diapause larvae to indoors cultured long-day *Sedum* foodplant, as a function of the age of the L1-egg larvae at time of activation. (Updated 22.9.79).

responses to a test foodplant-material. As can be seen from figure 1 the developmental response, of post-diapause L1 of the first generation, to a constant and optimal nutrient source is the same if activated in late autumn, in the winter or in the spring season. Since our data do not indicate a correlation between the age of the cold stored (dormant) L1 in the eggs at the time of activation, and the developmental response to optimal foodplants one can conclude that complete developmental competence of egg-larvae is established at termination of diapause in midwinter or earlier. Thereafter, but only under adequate conditions of cold-storage of eggs (or, as in nature, of cold-induced post-diapause dormancy), this state of competence is maintained for several months, i.e. until the time when the metabolic exhaustion of the egg larvae leads to a rather abrupt decline of both the egg hatchability and the viability of hatched L1 larvae.

4.2 Response of Apollo larvae to natural spring- and autumn foodplants

The response of *Apollo* larvae to the natural spring foodplant was tested in six experiments with *Sedum album* collected in the field between the middle of March and the end of May. This period of the year is characterized by a rapid increase of day-

length (from 11 hours on March 1 to 15 hrs 40 min on June 1). In March growth of *Sedum album* in the Jura region is accelerated and maximum growth rate is attained, depending on altitude and local climatic conditions, in May or June. This period also coincides essentially with the phase of intensive larval development of the Jura *Apollo* in its natural biotopes.

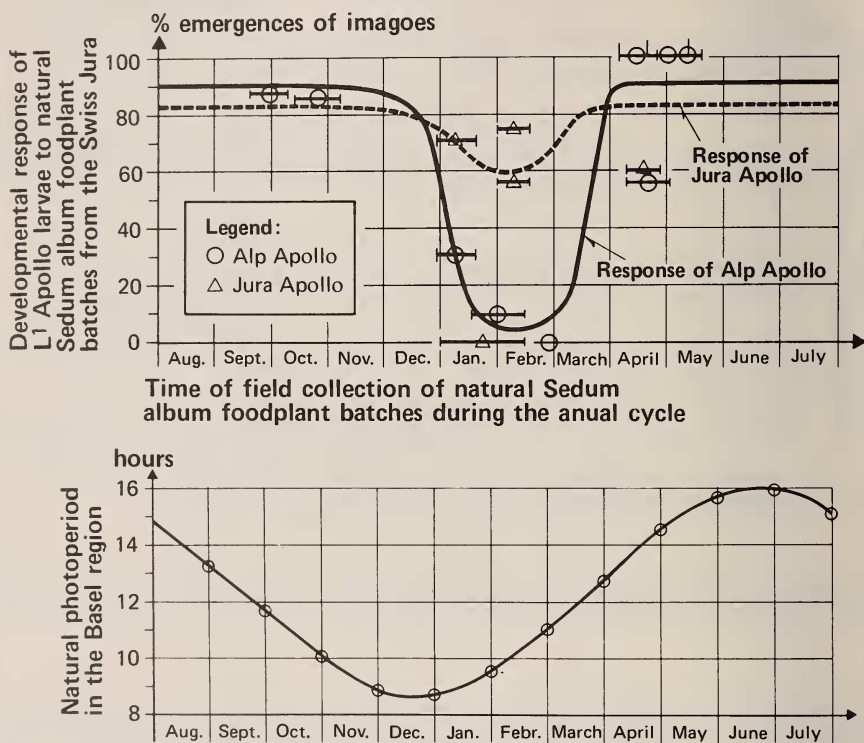


FIG. 2.

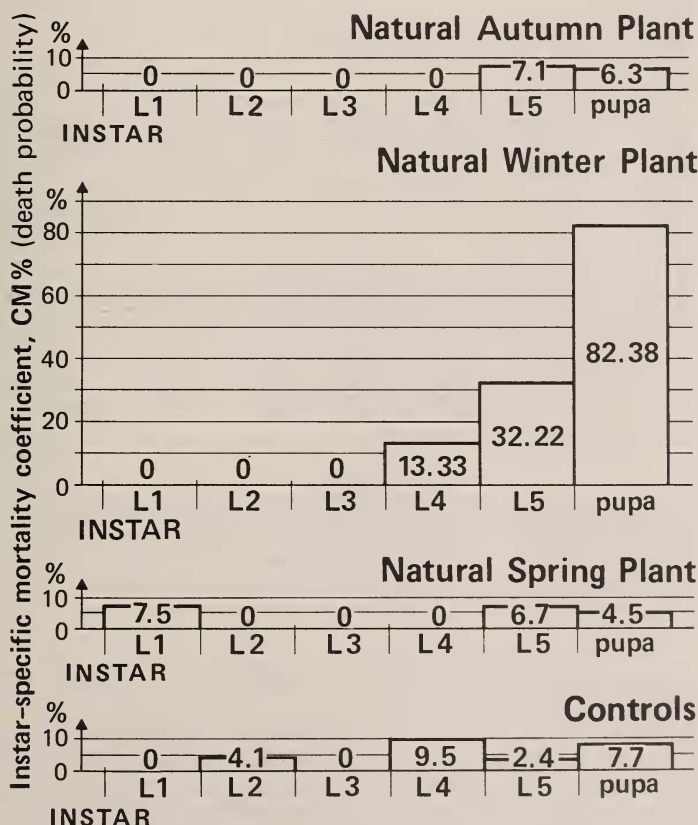
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Developmental-response characteristic of *Parnassius apollo* larvae, derived from the alpine- and Jura race, to (natural) *Sedum album* foodplant material collected periodically in the Swiss Jura region, and the corresponding natural photoperiodic cycle.

The results of the *Apollo* tests on natural spring *Sedum album* are listed in tables II and III and illustrated by figures 2 to 4. It is evident from these data that with respect to both the developmental response characteristic and the mortality profiles of *Apollo* larvae the spring foodplant is equivalent with the laboratory-bred LD (optimal) *Sedum* plant and yielded, in the average, 70% and 89% of viable imagoes derived from the Jura and alpine race respectively. The yield of adults with defective wing development averaged between 3% (alpine race) and 25% (Jura race). Insect mortality on spring plant tends toward zero during each of the first four larval instars and is augmented to a level of from 5% to 10% in L5 and in the pupal stage. There is apparently no indication of a differential response of the Jura- and alpine geographic races of *Parnassius*

apollo to natural spring-*Sedum*. Because of the difficulty of obtaining post-diapause egg larvae before midwinter, so far only two *Apollo* tests have been carried out with fresh foodplant material collected during the autumn months of September and October. However, the results obtained from these two experiments clearly indicate that the natural autumn foodplant is as efficient a nutrient source for *Apollo* larvae as are indoor-cultured LD and natural spring *Sedum* plants. The developmental response of larvae from the alpine race yielded an average of 87 % viable imagoes which included only 13 % of adults with incompletely unfolded wings. Moreover, the observed insect mortality profiles are conform with the profiles of insects grown on LD laboratory-bred plants or on natural spring plants.

ALPINE RACE



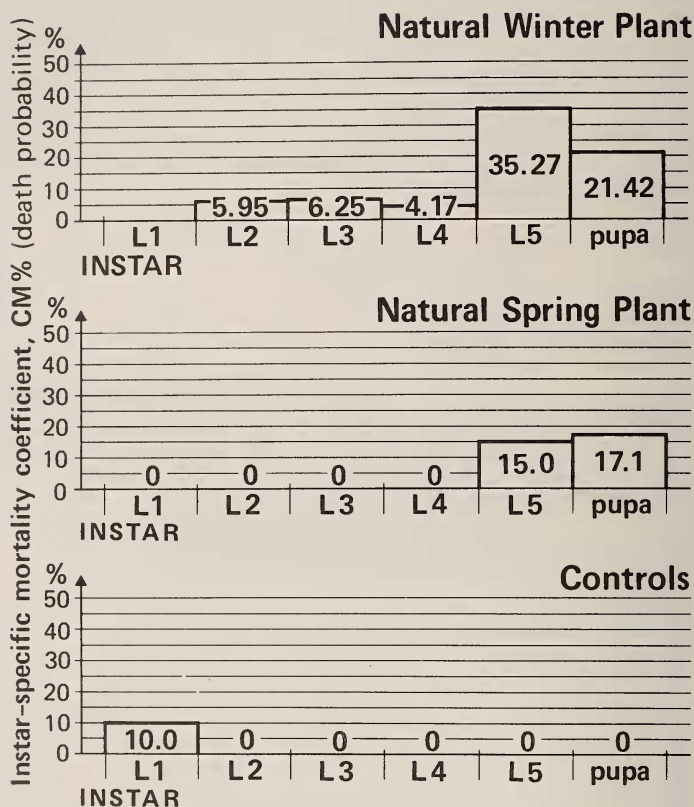
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FIG. 3.

Mortality profiles (average responses) of *Parnassius apollo* larvae, derived from the alpine race grown with (natural) *Sedum album* foodplant material collected periodically in the autumn-, winter- and spring season.

(Controls: Response to indoor-cultured long-day foodplant.)

JURA RACE



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FIG. 4.

Mortality profiles (average responses) of *Parnassius apollo* larvae, derived from the Jura race and grown with (natural) *Sedum album* foodplant material collected periodically in the winter- and spring season.

(Controls: Response to indoor-cultured long-day foodplant.)

4.3 Response of Apollo larvae to natural winter foodplants

When post-diapause L1 *Apollo* larvae were raised indoors in midwinter on *Sedum album* collected in snow-free localities of the Jura region during the months of January and February, a drastic increase of insect mortality was observed. In these winter experiments the total death rate reached frequently 100 % or nearly 100 %.

Instar-specific mortality on winter foodplant is highest either in the 5th larval instar or in the pupal stage, and total death rate is significantly smaller in larvae derived from the Jura race than in larvae derived from the alpine race (see tables II and III and figures 2-4). However, during the first four larval instars both, instar-specific death rates, and the developmental rates of survivors are essentially the same as those of larvae

grown on natural spring- or autumn plant or on laboratory-bred LD *Sedum*. In correspondence with the observed insect-mortality profiles the developmental response to natural winter *Sedum album* yielded only 13 % and 51 % of viable imagoes derived from the alpine and the Jura race respectively.

Insect hostility of the winter *Sedum* is demonstrated in a remarkable way when the *Apollo* larvae are reared in massculture rather than in single cultures. Probably because of reduced tolerance to latent microbial infection crowding leads to epidemic disease with fatal effects. On several occasions we have watched the rapid and total disintegration of crowded cultures at the stage when the majority of caterpillars had reached the 5th instar and were apparently ready for pupation. The mortality induced by the winter foodplant seems to be enhanced also by suboptimal light- and (or) temperature conditions. This is indicated by the results of some of our early tests which have been carried out under Neon light and with reduced temperature (19-20° C during the light cycles; 2° C or 19-20° C during the dark cycles).

5. DISCUSSION

The *Apollo*-tests with indoor-cultured LD *Sedum* as foodplant revealed no evidence of a reduced developmental competence of postdiapause L1 eclosing in mid-winter or earlier. Therefore, the abnormally high mortality rate of *Apollo*s reared in January and February on foodplants periodically collected outdoors in snow-free localities of the Jura region is due to the particular metabolic state of the natural *Sedum album* plant in mid-winter.

We have shown that during October and the first part of November, *Sedum album* growing in the natural biotopes is still an adequate or even optimal nutrient source for *Apollo* larvae. But at some time between mid November and the end of December — when the day length reaches its minimum of about 8.5 hours in our region — a change of the metabolic state is induced which, with some delay, changes the white stone crop plant, sometime in January, into a suboptimal nutrient source for *Apollo* larvae. These alterations apparently do not affect the foodplants capacities as an effective feeding attractant for *Apollo* larvae but exert frequently fatal effects on the insect, and those most often just before or during metamorphosis. The alpine *Apollo* race seems to be particularly sensitive to the metabolic alterations which distinguish the natural winter *Sedum* from LD foodplants growing under LD in nature or in the laboratory. As our data indicate the transition of the metabolic state of the hostplant takes place in the second half of March — that is at a time when the growth of *Sedum album* in the Jura region is accelerated under the influences of the rapidly increasing day length, mean light-intensity, and temperature. We do not know yet the factors which control these seasonal changes of plant metabolism but we suspect that the combined effects of short-day (1. signal) and cold i.e. frost (2. signal) are responsible for the induction of winter-foodplant inadequacy in nature.

Our preliminary experiments designed to demonstrate *direct* photoperiodic control of the alterations of the metabolic state of the foodplant have so far yielded inconclusive results. In these experiments (see table IV) L1 and L2 larvae were fed with fresh shoots from *Sedum album* hydrocultures, which had been grown at elevated temperatures 25-28° C) and under light intensities ranging from 8 000 to 35 000 Lux for at least 2 months either under LD conditions (19 hours light) or SD (8-10 hours light) conditions. As can be seen from table IV the response of the insect to cultured short-day foodplant

observed in different experiments is somewhat erratic and seems to depend largely on the particular hydroculture batch used as nutrient source. In a first series of four experiments the growth conditions of the foodplant had a pronounced effect on the insect: SD plant material (hydroculture batch KT1 practically always resulted in the death of the larvae, whereas a nearly 100 % survival was observed with LD plant food. In additional experiments with other hydroculture batches the effect of SD growth conditions on the *Apollo* larvae was less pronounced (hydroculture batches KT1A, KT3, KT4). However, pooling of the data of all these experiments shows an overall (average) 52 % increase of insect mortality due to the SD growth condition of the hydrocultured foodplant.

The nature of the biochemical plant factor(s) which is (or are) directly responsible for the insect hostility of natural winter-sedum foodplant is not known yet. However, we suspect that a secondary plant substance with insect toxicity (BERENBAUM 1978; BOWERS *et al.* 1967; DREYER *et al.*, in press) is involved ¹. If such a secondary compound is indeed involved its amount is likely to vary with the annual seasons, for example, accumulation of a photosensitive plant toxin will be favoured in winter, when light intensity is at its minimum, but may decline to insect-tolerable levels in spring. It is well known that *Sedum* plants and related Crassulaceans synthesise a variety of plant toxins, in varying amounts depending upon season and stage of plant development (SHNYAKING & MURZINA 1974). Many of these compounds are alkaloids, some of them are glycosides (KRASNOV *et al.* 1973) others are phenylpropane derivatives such as coumarin — which is apparently known to interfere with insect development (ROCKSTEIN 1978) — or oxycoumarins of the umbelliferone group (FRANK & HARTMANN 1963; GUMENYUK *et al.* 1971), or they are polyphenolic plant toxins such as myrecitine, quercitine, caempherol, gallic acid, arbutine and tannines (SHNYAKINA *et al.* 1974; SHNYAKINA 1974). Though practically every plant satisfies all the essential nutritional requirements (ROCKSTEIN 1978) of generalist and specialist herbivorous insects (minerals, amino acids, vitamins, carbohydrates, lipids and sterols) the possibility that cold-hardened winter *Sedum album* is nutritionally deficient for *Apollo* larvae (and perhaps also for other insects feeding on *Sedum*) cannot be excluded at present. It is known that in many plants the shortening of day-length together with mild frosts reduces the phospholipid content of cells. Since the lethal effects of natural winter *Sedum* manifest themselves late in larval life or during metamorphosis, a sterol or lipid deficiency may well be the chemical factor causing insect-hostility of the natural winter *Sedum*.

The ecological significance of the described insect-foodplant interaction cannot be fully assessed at present. It is clear, however, that the operational characteristics of this interaction are coevolutionary adaptations of host plant and insect to regions with cold winters and relatively warm summers. In this respect it is of interest to notice that none of 30 to 34 known species of Parnassiinae has crossed the tropical barriers from their palaearctic and nearctic habitats. The season during which insect-hostility of the natural foodplant is established or is at its annual maximum coincides with the natural cold-induced post-diapause dormancy period of the insect (see fig. 5). During this period, in the alpine biotopes, with their permanent snow cover and soil surface temperatures of around 0° C, the feeding activity of *Apollo* larvae (derived from early autumn- or mid-winter hatches) on the inadequate foodplant is probably negligible.

¹ Additional indications of the existence of secondary plant substances with high insect toxicity in *Sedum album* were recently obtained in tests with *Parnassius phoebus*, whose feeding preference is *Saxifraga aizoides* in the European Alps, but is a *Sedum* species in its nearctic habitats.

TABLE IV
Effect of short-day growth conditions of foodplant on *Parnassius apollo* larvae.

Experiment	Controls: Long-day <i>Sedum album</i>				Treatments: Short-day <i>Sedum album</i>				Short-day-plant effects:	
	Start instar	Hydro-culture	No.	Total deaths Decum	Decum %	Start instar	Hydro-culture	No.	Total deaths Decum	Decum %
PAB76EX01	L1	LT1	43	1	2.3	L1	KT1	24	24	100.0
PAB76EX02	L1	LT1	24	2	8.3	L1	KT1	26	26	100.0
PAB76EX04	L1	LT1	6	0	0.0	L1	KT1	10	10	100.0
PAB76EX03	L1	LT1	6 (3)	0 (0)	0.0	L2	KT1	10	10	100.0
PAB76EX07A	L1	*	9	2	22.2	L2	KT1A	3	0	0.0
PAB76EX07B	L1	*				L2	KT2	3	3	100.0
PAB76EX08	L1	*				L2	KT2	10	8	80.0
PAL77EX09	L1	LT3	8	3	37.5	L1	KT3	8	2	25.0
PAL77EX10	L1	LT4	10	3	33.3	L1	KT4	10	1	10.0
Mean										→ + 51.9

* Natural Autumn foodplant batches.

In the less elevated Jura biotopes, however, where the snow cover during the winter season is most often not permanent, the chance of exposure of young larvae to an inadequate foodplant is greater. Concurring with this increased likelihood of exposure, larvae derived from the Jura-*Apollo* race are apparently more resistant to the insect-hostile factors of the winter foodplant than are the larvae derived from the alpine race.

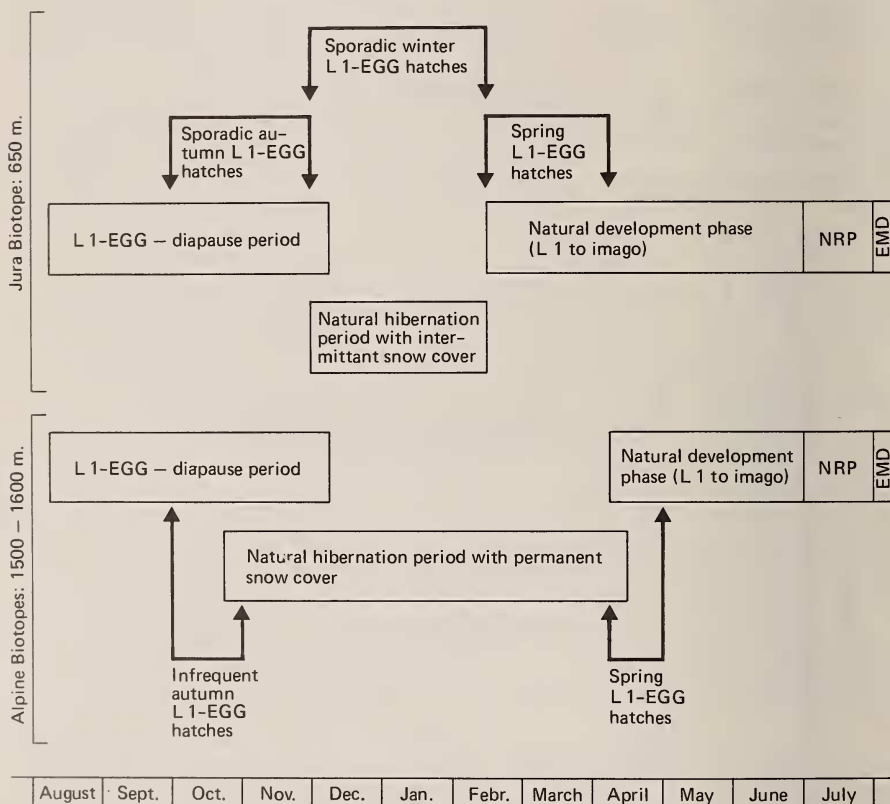


FIG. 5.

Approximate pattern of timing of the annual life cycle of *Parnassius apollo* in typical Jura- and alpine biotopes (NRP = natural reproduction period of insect; EMD = natural embryonic-development phase of insect).

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REFERENCES

- BERENBAUM, M. 1978. Toxicity of a furanocoumarin to armyworms: A case of biosynthetic escape from insect herbivores. *Science* 201: 532-533.
- BOWERS, W. S., T. OHTA, J. S. CLEERE and P. A. MARSELLA. 1967. Discovery of insect anti-juvenile hormones in plants. *Science* 193: 542-547.
- BRYK, F. 1935. Parnassiidae, Part II (Subfam. Parnassiinae): Das Tierreich. Vol. 65; Walter de Gruyter and Co., Berlin und Leipzig.
- DALY, H. V., J. T. DOYEN and P. R. EHRLICH. 1978. Introduction to Insect biology and diversity. *McGraw-Hill Book Company, New York*.
- DREYER, D. L., R. G. BINDER, B. G. CHAN, A. C. WAISS Jr., E. E. HARTWIG, G. L. BELAND, A. PINITOL (sous presse). Larval growth inhibitor for *Heliothis Zea* in Soybeans. *Experientia*.
- FRANCK, B., W. HARTMANN. 1963. The Sedum alkaloids. Abh. Deut. Akad. Wiss. Berlin Kl.-Chem. Geol. Biol. 1963 (4): 111-119, discussion 119.
- GUMENYUK, L. A., N. F. KOMISSARENKO, V. S. BATYUK, P. A. GNEDKOV. 1971. Coumarins of some species of the genus *Sempervivum* and *Sedum*. *Khim. Prir. Soedin.* 7 (3): 369 (Russ.).
- HARBORNE, J. B. 1977. Introduction to ecological biochemistry. *Academic Press, London, New York, San Francisco*.
- HESS, H. E., E. LANDOLT and R. HIRZEL. 1978. Flora der Schweiz und angrenzender Gebiete. *Birkhäuser Verlag, Basel und Stuttgart*.
- KRASNOV, E. A., T. G. KHORUZHAYA, L. V. PETROVA, R. A. AKSENOVA, and M. I. ZOTOVA. 1973. Chemicopharmalogical study of some representative Crassulaceae. *Usp. Izuch. Lek. Rast. Sib., Mater. Mezhvuz. Nauch. Konf.* 1973: 68-69 (Russ.).
- ROCKSTEIN, M. 1978. Biochemistry of Insects. *Academic Press, New York, San Francisco, London*.
- SHNYAKINA, G. P. 1973. Phytochemical study of stonecrops. *Usp. Izuch. Lek. Rast. Sib., Mater. Mezhvuz. Nauch. Konf.* 1973: 75-76 (Russ.).
- SHNYAKINA, G. P., N. B. MURZINA. 1974. Phenol compounds of some *Sedum* species of the Soviet-Far East and effects of their leaf extracts on the lipid metabolism of Rats. *Rastit. Resur.* 10 (3), 358-362 (Russ.).
- VINCE-PRUE, D. 1975. Photoperiodism in Plants. *MacGraw-Hill Book Company, London*.
- WALLACE, J. W. and R. L. MANSELL. 1975. Biochemical interaction between Plants and Insects. *Recent advances in Phytochemistry*, Vol. 10.

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